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Effect of Carbon Dioxide on Photorespiration[1]

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ABSTRACT

The isotopic CO_2 technique for measuring photorespiration was shown to be a valid technique for measuring the unidirectional inward and outward fluxes of CO_2 from a sunflower (*Helianthus annuus* L.) leaf in the light. The rate of photorespiration was decreased little as the CO_2 concentration was increased from 20 to 1,150 microliters per liter. This finding contradicts the widely held assumption that photorespiration is suppressed at high CO_2 concentrations. Some discussion regarding this apparent conflict is presented.

The only method that will measure photorespiration during steady-state photosynthesis is the isotopic CO_2 system devised by Ludwig and Canvin (15). Using this method, we showed that the rate of photorespiration was not altered over CO_2 concentrations from near zero to 300 $\mu l\ l^{-1}$ (16). Jackson and Volk (11), in discussing photorespiratory measurement with isotopic CO_2, made the following statement: "Assuming that each CO_2 species diffuses independently according to its own concentration gradient, there would be, immediately prior to the introduction of the labeled species, a net influx of $^{12}CO_2$ which is lower than the optimal influx." This would be because of the contribution of internal $^{12}CO_2$ production to the internal $^{12}CO_2$ concentration of the leaf. The internal production of "the isotopic species would be negligible immediately after their introduction, and hence the influx of $^{14}CO_2$ or $^{13}CO_2$ would exceed that for $^{12}CO_2$." This statement is based on the concept of net flux and implies that the observed differential between $^{14}CO_2$ uptake and $^{12}CO_2$ uptake is due to differences in the concentration gradients. If that conceptual analysis was correct then the observed differential uptake of isotopic species (15, 16) would have a physical basis and would not be of biological significance. It further implies that $^{14}CO_2$ uptake could be altered regardless of the external $^{12}CO_2$ concentration by changing the external $^{14}CO_2$ concentration. Inasmuch as the only method of measuring photorespiration during photosynthesis relies on differential uptake of isotopic CO_2 species it is not only important to establish the validity of the method but also to establish proper conceptual understanding. Here, we show that the concept of simultaneous unidirectional flux is more appropriate than the concept of net flux and that the method of using differential uptake of isotopic CO_2 species to estimate photorespiration is valid within the previously stated limitations (15). We further show that the rate of photorespiration was not greatly changed over CO_2 concentrations of 20 to 1,150 $\mu l\ l^{-1}$. A preliminary report of this work has appeared (4).

MATERIALS AND METHODS

Sunflower plants (*Helianthus annuus* L. var. CM90RR) were grown in plant growth chambers with a day/night temperature of 25/16 C and a light intensity of 400 $\mu E\ m^{-2}\ s^{-1}$ or with a day/night temperature of 25/20 C and a light intensity of 800 $\mu E\ m^{-2}\ s^{-1}$. Potting medium and nutrient supply were as previously described (7). Gas exchange measurements were performed on the fourth or fifth pair of leaves on 40- to 50-day-old plants.

Measurements of photorespiration were done as previously described (15) using either the system first described (15) or the later improved system (5). Measurements were performed with either an 11.5-ml or 33-ml ionization chamber (7). TPS,[2] APS, and PR were calculated as previously described (15).

Control of the $^{14}CO_2$ and CO_2 concentrations was achieved with steel bottles containing $^{14}CO_2$ and CO_2 mixtures (15) in combination with either gas-mixing pumps (1) (Wösthoff) or cylinders of CO_2-free compressed air.

The measurements were repeated six times but because of plant variability and variation in specific activity only two representative measurements are presented.

Transpiration measurements were made continuously to monitor stomatal aperture.

RESULTS AND DISCUSSION

If "each CO_2 species diffuses independently according to its own concentration gradient" in mixtures of $^{14}CO_2$ and CO_2 then the uptake of $^{14}CO_2$ does not reflect total CO_2 uptake but only the differences in concentration gradient. Jackson and Volk (11) stressed that "a crucial verification. . . could be to observe an actual accumulation of $^{12}CO_2$ molecules in the atmosphere simultaneously with net movement of the isotopic species inward" and commented on the difficulties of obtaining such measurements. As an alternative test, it is equally valid to show that $^{14}CO_2$ uptake is not solely dependent on its concentration gradient but is also affected by the ambient CO_2 concentration.

The $^{14}CO_2$ content ($\mu g\ l^{-1}$) of the supplied gas mixtures was held constant to maintain a constant concentration gradient for $^{14}CO_2$ and the CO_2 content was varied. In 21% O_2 it is clear that in such circumstances, $^{14}CO_2$ uptake was not constant but decreased as the CO_2 concentration was increased (Fig. 1). This means that the uptake of the two isotopic species was not independent and that the $^{14}CO_2$ uptake was reflecting the inward unidirectional flux of the total CO_2.

To investigate further the effect of the internal $[CO_2]$ on $^{14}CO_2$ and CO_2 uptake the leaves were pretreated with several CO_2 concentrations. Regardless of the preceding internal CO_2 concentrations the $^{14}CO_2/^{12}CO_2$ uptake ratio was the same after 15 s,

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[2] Abbreviations: TPS: true photosynthesis; APS: apparent photosynthesis; PR: photorespiration.

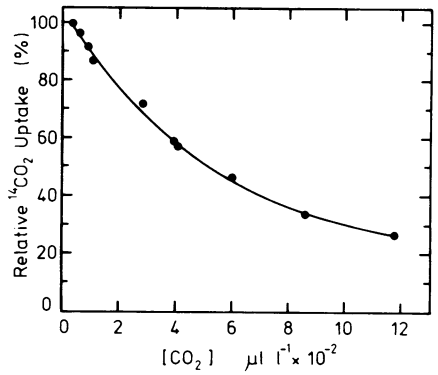

FIG. 1. Effect of CO_2 concentration (inlet) on the uptake of $^{14}CO_2$ by a sunflower leaf in the light. The $^{14}CO_2$ content ($\mu g\ ^{14}CO_2\ l^{-1}$) was held constant: the specific radioactivity ($^{14}CO_2$/total CO_2) decreases as CO_2 concentration increases. Measurements were performed on plants grown at the lower light intensity. Measurements were performed at 25 C and light intensity of 400 $\mu E\ m^{-2}\ s^{-1}$ using the 11.5-ml ionization chamber. Measurements of $^{14}CO_2$ uptake were made 15 s after the introduction of $^{14}CO_2$ to the leaf chamber.

which was the earliest time that a measurement could be obtained after the gas change (Table I). The rates of true and apparent photosynthesis were also identical in all treatments except when the leaves were pretreated with CO_2-free air. In that case both $^{14}CO_2$ and $^{12}CO_2$ uptake were depressed. The results of this series of tests either show that internal CO_2 concentrations have no effect on subsequent $^{14}CO_2$ or CO_2 uptake or that any effect of pretreatment on internal CO_2 concentration has been dissipated by the time the measurements could be made.

In the early time periods after supply of a mixture of isotopic CO_2 species there is a decrease in the specific radioactivity ($^{14}CO_2$/total CO_2) of the gas leaving the chamber compared to the specific radioactivity of the gas that entered the chamber. In their analysis of this observation Jackson and Volk (11) attributed this to greater uptake of $^{14}CO_2$ without concomitant uptake of the $^{12}CO_2$, implying that actual $^{12}CO_2$ evolution from the leaf into the atmosphere does not occur. From a net flux analysis their formulation is correct but it implies that $^{14}CO_2$ does not act as a tracer for the CO_2 in which it is supplied and also that an equilibrium situation where no net flux occurs is a static rather than dynamic situation with equal fluxes in both directions. Even when diffusion of CO_2 was the limiting factor in photosynthesis, the uptake of $^{14}CO_2$ was not independent of the concentration of CO_2 in which it was supplied (Fig. 1), *i.e.* its uptake was not a simple function of its diffusion gradient but rather it reflected the unidirectional inward flux of CO_2. It seems preferable to view the $^{14}CO_2$ uptake as estimating the total inward flux and that the dilution of specific radioactivity that occurs in the plant chamber is due to a unidirectional outward flux of $^{12}CO_2$. Thus, even when the net flux of $^{12}CO_2$ is inward the outward flux can be detected when a second isotopic species ($^{14}CO_2$) is present. It is not necessary as Jackson and Volk (11) proposed to show net flux of $^{12}CO_2$ from the leaves.

The above results and arguments support our previous interpretations (15) of gas fluxes as measured with isotopic CO_2 species, *i.e.* $^{14}CO_2$ uptake and specific radioactivity allow one to determine the total inward flux of CO_2 from the atmosphere and this is a minimum estimate of true photosynthesis; the $^{12}CO_2$ measurement represents the net flux of CO_2 or apparent photosynthesis and the difference between these two must represent the outward flux of CO_2 or photorespiration. In other words TPS − APS = PR. Of course TPS is still underestimated because of refixation inside the leaf and also because the specific radioactivity of the CO_2 at the site of fixation cannot be measured, but there is little doubt that it is lower than that measured in the external atmosphere. Because TPS is underestimated PR will also be underestimated but these are the only estimates of photorespiration that can be obtained during steady-state photosynthesis.

The problem of specific radioactivity ($^{14}CO_2$/total CO_2) at the site of fixation is the major deficiency of the isotopic CO_2 method as internal refixation is part of this problem. If one could obtain a better estimate of the specific radioactivity at the site of fixation one could obtain a better estimate of TPS and PR. We have already shown that up to a $[CO_2]$ of 300 $\mu l\ l^{-1}$, photorespiration was not affected by the external CO_2 concentration (16). In this range of CO_2 concentrations where CO_2 diffusion limits the overall process there would be no accumulation of CO_2 at the site of fixation (3) and thus there is maximum dilution of incoming CO_2 by the CO_2 that is internally produced. As the external CO_2 concentration is raised, there would be accumulation of CO_2 from the supplied gas at the site of fixation (3, 4). There would then be less dilution of the supplied specific activity. Thus, the specific radioactivity of the CO_2 in the leaf chamber is a better estimate of the specific radioactivity at the site of fixation and better estimates of TPS and PR would be obtained.

The measurements of TPS, APS, and PR using the $^{14}CO_2/CO_2$ technique at 25 C are shown in Figure 2 and at 31 C in Figure 3. At 25 C (Fig. 2) photorespiration increased as the CO_2 concentration was increased. This may not be due to an actual increase in photorespiration but rather due to a better estimate of photorespiration as outlined in the above paragraph. At 31 C (Fig. 3) there was a small decrease in photorespiration as the CO_2 concentration was increased from 20 to 1,150 $\mu l\ l^{-1}$. On the evidence presented, we cannot claim that photorespiration increases as the CO_2 concentration increases but we can state with certainty that photorespiration does not decrease much if at all as the CO_2 concentration is increased from near zero to 1,150 $\mu l\ l^{-1}$. Fock and Przybylla (8)

Table I. The effect of pretreatments (30 min) of different CO_2 concentrations on the subsequent uptake of $^{14}CO_2$ and CO_2.

Measurements were performed on sunflower plants grown at the lower light intensity. Measurements were performed at 25°, 400 μeinsteins. m^{-2}. sec^{-1} using the 11.5 ml ionization chamber. Measurement of TPS was made 15 sec after the introduction of $^{14}CO_2$ to the leaf.

Pretreatment $[CO_2]$ $\mu l.l^{-1}$	Treatment $[CO_2]$ $\mu l.l^{-1}$	APS $mg.dm^{-2}.hr^{-1}$	TPS $mg.dm^{-2}.hr^{-1}$	$^{14}C/^{12}C$ uptake ratio x 10^2
400	300	17.60	19.56	3.27
0	342	10.90	12.05	3.24
300	335	17.60	20.93	3.56
85	335	18.30	21.23	3.32
700	333	16.90	20.77	3.49
1000	335	17.29	20.44	3.32

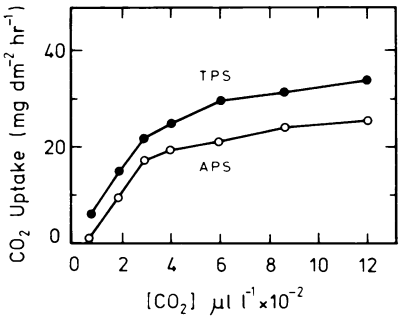

FIG. 2. Effect of CO_2 concentration (inlet) on true (TPS) and apparent photosynthesis (APS) of sunflower leaves. Plants and measuring conditions as for Figure 1.

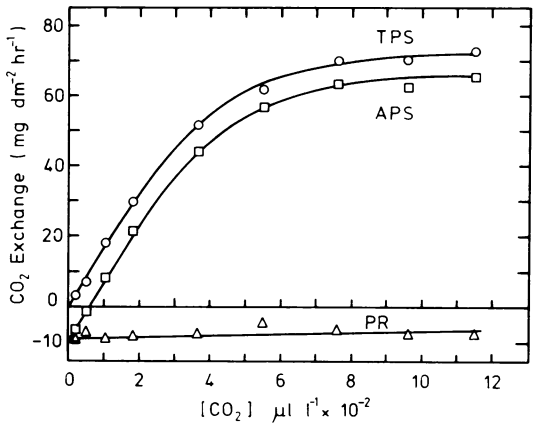

FIG. 3. Effect of CO_2 concentrations (average in plant chamber) on true (TPS) and apparent photosynthesis (APS) and photorespiration (PR) of sunflower leaves. Plants were grown at the higher light intensity. Measurements were performed at 31.2 C, and light intensity of 1,350 µE m^{-2} s^{-1} using the 33-ml ionization chamber. Stomatal resistance of the leaf was between 0.67 and 0.79 s cm^{-1} over the entire CO_2 concentration range. Measurement of TPS was made 20 s after the introduction of $^{14}CO_2$ to the leaf chamber.

also found no decrease in photorespiration when the CO_2 concentration was increased.

These findings apparently contradict the widely held assumption that photorespiration is suppressed at high CO_2 concentrations. It is not possible to support fully, with existing evidence, any reconciliation of these results with an extensive literature of indirect evidence which supports the above assumption (6, 20). It is useful to bring attention to some recent results that question the interpretations that have been drawn from those earlier experiments. In the interests of brevity only two features of the earlier work will be discussed.

It has been proposed that both photorespiration and the effect of O_2 on photosynthesis are due solely to the reaction of O_2 with ribulose-1,5-bisphosphate carboxylase (RuBPCase) (6, 17). As the CO_2 concentration is increased the effect of O_2 on apparent photosynthesis decreases until, at high CO_2 concentrations, changes in the O_2 concentration have little effect on the rate of photosynthesis (6, 20). At this point it is also assumed that photorespiration is suppressed. There is no doubt that, *in vitro*, CO_2 competitively inhibits oxygenase activity of RuBPCase (2) but is this also the explanation for the apparent lack of effect of O_2 on *in vivo* photosynthesis at high CO_2 levels (13)? Recent reports (5, 14, 19) show that at high CO_2 concentrations, where the capacity of the leaf for CO_2 fixation is fully taxed, there is an inhibition of photosynthesis when the O_2 concentration is decreased from 21 to 2%. These data suggest that at high CO_2 concentrations the capacity of the leaf to fix CO_2 in 2% O_2 is less, or, after an adaptation period, only equal to the capacity of the leaf to fix CO_2 in 21% O_2. If the capacity of the leaf to fix CO_2 has already been reached, the elimination of photorespiration in low O_2 cannot be expressed as an increase in the rate of photosynthesis. Photorespiration could continue in 21% O_2 at high CO_2 concentrations but it would not be detected from measurement of apparent photosynthesis in lowered O_2 concentrations.

The other feature that will be discussed is the decrease in glycolate accumulation in the presence of an inhibitor of glycolate oxidation when the CO_2 concentration is increased (6, 20). Considerable evidence has been presented (20) that the inhibitor, α-hydroxy-2-pyridine methane sulfonate, blocks the glycolate pathway and hence photorespiration. It is believed that under these circumstances glycolate accumulates because its oxidation is blocked but its production rate is not affected. The decrease in glycolate accumulation, then, as the CO_2 concentration is increased, is due to decreased glycolate synthesis and this decrease should result in decreased photorespiration according to our current understanding of the relationship between glycolate metabolism and photorespiration. Recent results (12, 18) using another inhibitor (butyl hydroxy butynoate) of glycolate oxidation, however, show that, even when glycolate accumulates, CO_2 evolution or photorespiration is not greatly affected. Thus, the proposal that photorespiration is inhibited by a glycolate oxidation inhibitor was not upheld by these experiments and it would seem that when the glycolate pathway is blocked, glycolate may still be metabolized to CO_2 by an alternative mechanism (9, 10, 21). If glycolate can still be metabolized in the presence of a glycolate oxidase inhibitor then the accumulation of glycolate may not reflect only its rate of production but rather an equilibrium between production and comsumption. If this is so, a decrease in glycolate accumulation at higher CO_2 concentrations may not lead to a decrease in photorespiration.

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